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Ovine Enzootic Abortion (OEA): a comparison of antibody responses in vaccinated and naturally-infected swiss sheep over a two year period

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Abstract

Background

Preventiona ndc ontrolof ovi nee nzootica bortion(OEA) canbe achievedb y applicationof a liveva ccine. Int hiss tudy,f ives heepf locksw ithdi fferentv accinationa ndi nfections tatus weres erologicallyt ested usinga c ompetitivee nzyme-linkedi mmunosorbenta ssay (cELISA) specificf or *Chlamydophila(Cp.)abor tus* over at wo-yeart imepe riod.

Results

Sheepi nF lockA w ithr ecentO EAhi storyha dhi gha ntibodyva luesa fterv accinations imilar toF lockC withna tural *Cp.abor tus*i nfections. In contrast,O EAs erologyn egatives heep (FlockE)s howedi ndividuala nimal-specifici mmunoreactionsa fterv accination.A ntibody levelsof va ccinatede wesi nF lockB ranged from negativet opos itivet woa ndt hree years afterv accination,r espectively.P ositivea ntibodyv aluesi nt hene gative controlF lockD (withoutO EAor va ccination)a repr obablydu et o asymptomatici ntestinali nfectionsw ith *Cp. abortus*.E xcretionof t hea ttenuateds trainof *Cp. abortus*us edi nt hel iveva ccinet hrought he eyew asnot obs ervedi n vaccinateda nimalsof FlockE .

Conclusions

Thef indingso four s tudyi ndicatet hat,us ings erology,nodi stinctionc anb em adebe tween vaccinateda ndna turally infecteds heep.A sa result,c onfirmationof a n egativeO EAs tatusi n vaccinateda nimalsb ys erologyc annotbe de termined.

Background

Chlamydophilaabor tus(formerlyC hlamydiaps ittacis erotype1)i st hem ostc ommon infectiousba cteriac ausinga bortioni ns mallr uminantsi nS witzerlandw ith apr eviouss tudy demonstratingt hat39% oft hee xamineda bortionsi ns heepa nd23% i n goatsw ere causedb y thisa gent[1]. Int heS wissc antonof G raubünden,a m ountainousr egioni nt hec ountries' east,t hee conomicl ossesa ssociatedw ithovi nee nzootica bortion(OEA)a res ignificantly highert hani not herc antons[2].

*Cp.abor tus*i sg enerallyi ntroducedi ntoi mmunologicallyn aïvef locksb y a latentlyi nfected animalw itht hea gentbe ings ubsequentlyt ransmittedf roma bortinge wesv ias heddingo f largea mountsof i nfectious *Chlamydia*i nt hef oetalm embranes andi nva ginaldi scharges[3]. Inn ewlyi nfectedf locks, upt o30% of e wesm aya borti nt hel astt rimesterof ge stationor givebi rtht ow eakor d eadl ambs.A ftera bortion,e wesi nt hese flocksm ayde velopa protectivei mmunity.S ubsequent yearlyl ossesi ne ndemicallyi nfected flocksm ayde creaset o al owerl evel(eg.5- 10%)w iths heepe itherbor ni ntot hef lockor ne wlyi ntroduceda nimals likelyt os uffera bortions duringt heiri nitialpr egnancies[4,5].

Preventiona ndc ontrolof O EAi sa chievedb yva ccinationa nd/ort reatmentw ith oxytetracyclines[6].T wova ccines againstc hlamydiala bortiona rel icensedi nS witzerlandb y theF ederalV eterinaryO ffice(FVO)i nB erne. Thef irstof t hese available wasa ne gg-grown, formalin-inactivated, whole-organismva ccine(OvaxC lamidia,F atro, Italy) whichr educes thei ncidenceo fa bortion inva ccinatedh erdsbut n otc ompletely [7-10].S inceD ecember 2002,a na virulent,t emperature-sensitive,l ivec hlamydiava ccine (Ovilis®Enzovax,I ntervet, TheN etherlands),w hich ism arketedt oi nduces trongl ong-lastingpr otection,ha sbe enm ade commerciallya vailablei nS witzerland.T hea ttenuateds train1B , whichf ormst heba sisof t his vaccine, wasobt ained fromt hevi rulent *Cp.abor tus*s trainA B7b yni trosoguanidine mutagenesis[11-13].

In2005, as mallpi lots tudyw asunde rtakent ode terminei fa dministrationof va ccinest o protects heep flocksf romO EAw ouldr esulti na ntibodyl evelsi nt hec omplement-fixation test(CFT)a ndi nt he competitivee nzyme-linked immunosorbenta ssay (cELISA)t estss imilar tot hosef ollowingna turali nfection[14].A fterva ccinationw itht hei nactivatedva ccine(Ovax Clamidia)onl yone s heepde velopeda d etectable antibodyr esponse. In contrast,va ccination witht hea ttenuatedl ivev accine (Ovilis®Enzovax) resultedi nde tectablea ntibodyt itersi na ll testeds heep.

Thea imof t hiss tudyi st oi nvestigate al argernu mberof s heepove r at wo-yearp eriodi nt he fieldt oc ompare flock-levelE LISAr esponsesb etween(a)va ccinated(liveva ccine),(b) naturallyi nfected and(c)non -infecteds heepf locks. Itw asa nticipatedt hatt hef ollowup studyof t hehum oralr esponsesc ouldpos siblydi scriminatebe tweenv accinateda ndna turally OEA-infecteds heep.A n additionalo bjectiveof t hes tudyw ast oa ttemptt ode tectc hlamydiae and/ort hea ttenuateds trainof *Cp.abor tus*us edi nt hel iveva ccinei n conjunctivals wabsof sheep.

Results

Serological results and abortion cases

cELISAc lassifications(frequency andpr oportionpos itive),m ediant itera ndr espective range ofpos itivec lassifieds heepi nf locksA , B,C ,D a ndE ove rt hef ourdi fferenti nvestigation datesa res howni nT able 1.T hec omparisonbe tweenva ccinateda ndnon -vaccinated animals inF lockB andE i ss howni nT able2.F igure1s howst het iterr anges (boxpl ots)of a ll examineds heepi nt hef ivef locksove rt hef ouri nvestigationda tes.

Alle wes(n=15)of FlockA w eres erologicallypos itivea fterva ccinations howinga hi gh mediana ntibodyv alueof 91.7% .T hem edian antibodyl evelof pos itives heep(n=13)

decreasedm arginallyt o 86.6%i na utumn2005. Ins pring2006a nd autumn2006,t he seroprevalencei nt hef lockw as73% (n=11).T he mediana ntibodyv alueof t hepos itives heep was81.3% (spring2006) a nd82.3% (autumn200 6).

Ins pring2005,t wo yearsa ftert he firstva ccination,s ixout of 14va ccinateds heepi nF lock B hada pos itives erologicalr esult(mediana ntibody value62.9%),w hereast woout of 12non - vaccinateds heepi nt hes amef lockw erepos itive. Thenum berof pos itives heepde creasedt o threea ndt woi nt hev accinatedgr oup(n=14)i na utumn2005a nds pring20 06,r espectively. Int heno n-vaccinated group,one s heept estedpos itivei na utumn2005,but none i ns pring 2006. In autumn2006,t henum berof p ositives heepi ncreasedi nt heva ccinated(n=6)a nd non-vaccinated(n=2) group,a lthougha bortionsw erenot r eported.T hem eana ntibodyv alues int het wog roupsw erec omparable,bot hbe ings lightly greatert han60 %.

FlockC (naturallyi nfectedf lock)s erved ast hep ositivec ontrol.T hes eroprevalencei ns heep ins pring2005w ashi gh at82% (n=14).T hem ediana ntibodyv aluei nt he positiveg roupw as 82.9%.T hes eroprevalencer emainedc ontinuously high(76%-88%)du ringt hew holes tudy perioda ndm edian antibodyva luesi npos itives heepw erea bove70 %. In autumn2005 newbornl ambsw erel argelyn egativea ndh ada s ignificantlyl owerm ediana ntibodyva lue thanol dere wes(KruskalW allist est,p< 0.05)(datanot s hown).T he ewew itht hec onfirmed chlamydiala bortioni ns pring2005h adpos itivea ntibodyl evelsf ort her emainings ampling periodc omparablet ot he othera nimalsi nt hef lock(50.5%-77%).T hes eroprevalencei n goatsa fter confirmed chlamydial abortioni na llf oura nimalsi ns pring2005 w as100% (n=4) witha hi ghm edian antibodyva lueof 91.6% (datanot s hown). Inc ontrastt ot hes heep,a ll goatsr emaineds erologicallypos itivew ithve ryhi gha ntibodyva lues(71.2%-97.5%)ov ert he wholet estingpe riod(datanot s hown).

FlockD s erveda st hene gative controlf ort hiss tudy.D espitet his,21% (n=13)of t hee wes showedpos itiver esultsi ns pring2005,w hereas4 4%(n=28)o ft hee wesh adne gative serologicalr esultsa nd35 %(n=22)o fa nimalss howedque stionabler eadings.T hem edian

antibodyva luesof t hepo sitivea nimalsw ere69.5 %.H alfa yearl ater,i na utumn2005,21 animalsc ontinuedt obe s erologicallypos itive. Ins pring2006,t hes eroprevalencei ncreasedt o 46%,w hereast hem eana ntibodyva luesof t hepos itivea nimalsw ere comparablet os pring anda utum2005(around 69%). Ina utumn2006,t henum berof s erologicallypos itivee wes decreasedt o30% (n=19),w hereast hem ean antibodyva lueof pos itives heepi ncreasedt o 74.3%.

Priort ova ccinationi ns pring2005,onl yone animali nF lockE w aspos itivei nt he vaccination group (antibodyva lue61.4%),w hereas25s heep(50%)w ere positivei nt henon-vaccinated group(n=50).A ll13s heepof t heva ccinated groupw eres erologicallyn egativei n autumn2005a ndt herefores electedf orv accinationi nw inter2005.T heno n-vaccinated group showeds eroprevalences between38- 48%f rom autumn2005t o2006a ndm ediana ntibody valuesof pos itivea nimalsw ere consistentlybe tween80.4 -82.2%. Inc omparisont o vaccinateds heepi n FlockA ,none of t he animalsva ccinatedi nw inter200 5w ere serologicallypos itivei ns pring2006. In autumn2006,one e weh ada pos itivea ntibodyva lue of73.2% ,w hereast heot her12va ccinateds heep hadne gative(n=6)or qu estionableva lues (n=6).

Statistical comparison of mean titers

In flocksA (alla nimalsva ccinated),C a ndD (no animalsi nbot hf locksva ccinated,F igure 1),di fferencesi nt iterva luesbe tweent hes amplingpe riodsw erea lwayshi ghlys ignificanti n theR MA NOVAm odel(p< 0.01) . In Flock B,w itha va ccinationda tebe tweens ampling periods2a nd3,bot hva ccinations tatusa nda ni nteractiont ermbe tween vaccinationa ndvi sit weres tatisticallys ignificant(p< 0.05) . InF lockE ,i nw hichva ccinationt ookpl acebe foret he firsts ampling,bot hm aine ffectsw eres ignificant(time:p< 0.05; va ccinations tatus:p< 0.01),w hilet hei nteractiont ermw asnot .

PCR results of eye swabs

In FlockE ,118 conjunctivals wabsw erec ollected before applicationof t he liveva ccinei n autumn2005.N oobvi ouss ignsof oc ulars urface diseasess ucha s conjunctivitisa ndke ratitis wereobs ervedi n anya nimal. IGS-SP CRs creeningde tected22s amplest hatw erepos itive forc hlamydialD NA.S equencingof t heseP CRpr oductsi dentified18s amplest hats hared greatert han98% s equences imilarityt o *Cp.abor tus*[GenBank:C R848038.1].O nes ample eachw asr evealedt ob e positivef or *Cp.pe corum*[GenBank:C PU68434]a nd *Cp.f elis* [GenBank:A P006861.1].T hei dentityof t wos amplesc ouldnot be de termined.
Fivem onthsa fterv accination,i ns pring2006,11 8e yes wabsw eres ampledi nt hes amef lock. 12s amplesw eret estedp ositiveb yt he IGS-SP CRbut a llw eref romnon- vaccinatede wes.O f theses amples,5/ 12w erepos itivef or *Cp.abor tus*[GenBank:C R848038.1]w hilet hreew ere positivef or *Cp.pe corum*[GenBank:C PU68434].T hei dentityof fours amplesc ouldnot be determined.N oneo ft he vaccinateds heeps howeda pos itive IGS-SP CRr esulta ndi tw as concludedt hatnoe xcretionof t heva ccines trainh adoc curred.

Discussion

Thiss tudyr epresentst he firstl ongterm chlamydials erologicals tudy comparingva ccinated andnon- vaccinated flocksi nS witzerland.T hei nvestigationsw ereunde rtakeni nt hec anton Graubünden, wherenum erousc hlamydiala bortionc asesi ns heepw erepr eviouslyr eported [1]a ndt hehi ghests eroprevalence(43%)f or *Cp.a bortus* inS wissc antons wasobs erved[2]. Ther esultsobt ainedf romt hiss tudyc onfirmt he previousobs ervationsof thepi lots tudy[14] thats erology (cELISA)c annotbe us edt odi stinguishbe tweens heepva ccinatedw itht hel ive attenuatedva ccine andn aturally-infecteds heep. Thea ntibodyv aluer angei nt her ecently vaccinatedF lock Aw as comparablet o FlockC i nw hicha cutei nfectionso f *Cp.abor tus* occured att hes amet ime. In FlockA ,v eryhi gha ntibodyl evels(around90 %)w erevi siblei n

everyva ccinateds heep (n=15),w hereas antibody levelsof s heepi nt hep reviouspi lots tudy weres omewhatl ower(around60%)21da yspos t vaccination[14].A sc hlamydiala bortion wasr eportedi nF lock Ai nt hepa st,s heepc ouldh avebe ena lreadys erologicallypos itive beforev accinationa ndt heve ryhi gha ntibodyl evelsc ouldr epresenta nove rlayo fbot h abortiona ndva ccine-associateda ntibodyva lues. Them eana ntibodyva lueof pos itive animalsde creasedi nbot hf locks(Aa ndC) from spring2005t os pring20 06.A c hlamydial abortionw asdi agnosedi none g oatf rom FlockC i na utumn2006e xplainingt hei ncreasing seroprevalencea nda ntibodyva luei nt hisg roupo fa nimalsa tt hatt ime.T hea ntibodyv alues int heg oatsof FlockC a ftera na cutei nfectionw ith *Cp.abor tus*w erehi ghera ndpe rsisted ata veryhi ghl evel(80t o90 %)ove rt heo bservationpe riodc omparedt ot hes ituationi ns heep. Noc orrelationw ithpr otectionw ass eenhow evera sa c hlamydiala bortiono ccurredi na seropositiveg oatw hich hadpr eviouslya borted. Thisobs ervationw asa lsom adei not her goat flocksi nc antonG raubünden(R.T homa,pe rsonalc ommunication).G oatst reatedw itht he liveva ccine alsoa borted. In general,i ti sknow nt hati f *Chlamydiae*a rei ntroducedi na n aive flock,t hel ossesa rem uchhi gheri n goats(60%)t hani ns heep(30%).T hedi fferences between goatsa nds heep arec onsistentw ithpr eviousr ecords andt oda ter emainune xplained [15,16].

Antibodyl evelsof v accinatede wesof F lockB ranged fromne gativet opos itivet woa ndt hree yearsa fterv accination,r espectively.Q uestionablea ntibodyl evelsa ree ithera ttributedt o undiagnosed *Cp.pe corum*i nfections[17]or a rep ossiblydu et ot heva ccinationi ns pring 2003. In as imilars ituationt ot he naturallyi nfecteds heep(FlockC), as lowde creaseo f antibodyva luesw asobs ervedove rt hes amplingp eriod.T hisobs ervations tronglys uggests thats erology (cELISA)c annotbe us edt odi stinguishbe tweens heepva ccinatedw itht hel ive attenuatedva ccine andn aturally-infecteds heepa sa nticipatedi nt hepr eviouspi lots tudy [14]. Asa di rect consequencet ot his,t hec onfirmationof ne gativeO EAs tatusi nva ccinated animalsb ys erology cannotbe m ade.T hisi sunf ortunatea sr eliable confirmationi si mportant

ifa na batementof O EAt hrough assemblyof O EA-freef locksi st obe pe rformeda s undertakenb yt heS heep andG oatH ealthS chemesi nE nglanda ndW ales andt heP remium HealthS chemei nS cotland.

Positivea ntibodyv alues havebe enobs ervedi nt hene gative controlf lock(FlockD), which hadnot be enva ccinated andw asf ree fromc hlamydiala bortion.A ne xplanationf ort he observationsof a ni ncreasinga ntibodyva lue amongstt hisf locki st hatt he animalsm ayh ave asymptomatici ntestinali nfectionsw ith *Cp.abortus*a spr esumedi npr eviouss tudies[17,19]. Ana lternatives cenarioi st hatt hee wesw erei nfectedw itha l essvi rulents trainof *Cp.abortus*, whichpr ovokess eroconversionbut noa bortion[17,20].F luctuationsi nt hea ntibodyl evels couldbe t her esultof b acterials heddingdur ingoe strusw hichpr ovokesa ni nductionof antibodyl evelsw ithoutc ausinga bortion [21,22]. Unfortunately,l ittlei ss tillknow na tt his timea boutt hea bilityof *Cp.abor tus*t ope rsisti n animals(andt hea natomicall ocationof t his persistenti nfection)c omparedt oot her chlamydials pecies,w hichr equire morei nvestigations.

In FlockE ,t hes erologicalr eactionof 13s electedva ccinateds heep andt he50non- vaccinated sheepi nt hef lockw ase valuated.S urprisingly andi nc ontrastt ot heobs ervationsi nt he previouspi lots tudy[14]a ndi nt het wova ccinatedf locksA a nd B,s ixof 13va ccinateds heep ofF lockE s howednos eroconversione ightm onthsa fterva ccination.O nly onee weh ada positives erologicalr esult(73.2%),c omparablet o theva ccinateds heepo fF lockA a ndt he naturally OEA-infecteds heepof FlockC .T her emainings ixe wesha dqu estionablea ntibody levels.T hepr imarydi fferenceb etweena nimalsi nf locksA a ndE w ast he highva riabilityof antibodyl evelsi nva ccinateda nimals.T hese resultss uggestt hati ndividuali mmunoreactions betweens heepc anva ry considerably.

Samplingof c onjunctivals wabsf roms heepi nF lockE w aspe rformedt ode tecta nd compare thepr esenceof chlamydialD NAbe fore anda fter vaccination. Furthermore,a pos sible

excretionof t heva ccinet hrought hee ye couldbe screenedw itht hisa pproach.A lthough chlamydiaew ere frequentlyde tectedb yP CRi nc onjunctivals wabsof s heep,t hea ttenuated strainof *Cp.abor tus*us edi nt hel iveva ccine was notde tectedi ns wabs collectedf rom vaccinateds heep.T hei ncidenceo f *Cp.abor tus*a nd *Cp.pe corum*a nde ven *C.s uis*i n clinicallyhe althynon -vaccinateds heepw asp reviouslyobs ervedi na r ecents tudy [23].T he significanceof t hispos siblene wm odeof t ransmissionf orO EAne edsf urtheri nvestigation.

Conclusions

Thef indingsi nour s tudys tronglys uggestt hats erology(cELISA) cannotb eus edt o distinguishbe tweens heepva ccinatedw itht hel ivea ttenuatedv accinea nd naturally-infected sheep.T he courseo fa ntibodyl evels,ne vertheless,c anva ryb etweeni ndividuala nimalsa nd flocks.C omparedt os heep,g oatsdi splayedhi ghera ntibodyl evels,w hichp ersistove ra longert imep eriodbut do not c orrelatew ithpr otection.T hea ttenuateds trainof *Cp.abor tus* usedi nt hel iveva ccine wasnot de tectedi n eyes wabsc ollected fromva ccinateds heep.

Methods

Flock details

Fivedi fferents heepf locksi nt hec antonG raubündenw eref ollowedov era t wo-yearp eriod withf ourf lockvi sits.T hesef ivef locksw erea vailablef ort hes tudyi ns pring200 5t hrough an establishedc ollaboration withve terinary authoritiesi nt hec antonG raubunden.

Duet oc onstantt urnover ine achf lock (i.e.s laughteringo fol dor s icke wes,bi rthof l ambs, introductionof ne wa nimals)t henum berof animalst esteda llf ourt imesw asm uchl owert han

thenum berof i ndividual sheepi nt hef lock.D etailsont hef ivet estedf locks(A,B ,C ,D and E)ove rt he fouri nvestigationda tes(spring2005/ 06,a utumn2005/ 06)a rep rovidedi nT able 3.B riefly, animalsof F lockA w erea vailablef ors erologicalt estinga fterva ccinationof 15 sheepi ns pring2005.H istoryof chlamydiala bortioni na utumn2004w asr eported,but none oft he15s heepi nt hes tudys uffereda na bortiondur ingt he examinationpe riod.E wes(n=14) ofF lock Bw erev accinatedi ns pring2003w itht hel iveva ccinebe causeof a c hlamydial abortionout breaki nt he vicinityof t hisf lock. Beforea nda fterv accination,noa bortionsdue to *Cp.abor tus*oc curreda nd,a sa r esult,t heow ner abandoned ava ccination boostert wo years later.A ccesst ot hisf lockw aspos siblei ns pring2 005.F lockC ha da n averageof 11 goats overt hef ouri nvestigationda tes,of w hich fourw erea vailablef or repeatedt estingdur ingt he fours amplingp eriods,b utt her esultsw erenot i ncludedi nt heove ralls tatisticalc alculations ofF lockC .F lockC ha d confirmedc hlamydiala bortionsi na utumn2004(unknownnum ber ofa nimals)a nds pring20 05(onee wea ndf our goats).N of urther chlamydiala bortions occurredi nt hisf locka fters pring2005.A nimals sufferingf roma bortions weret estedf our timesdur ingt hes tudy.F lockD r epresentedt hen egative controlf lock asnoa bortionor vaccinationoc cureddu ringt hes tudype riod.S heepi nt hisf locks pents ummert ogetherw itha flockt hatha dr eportsof chlamydiala bortioni nt hepa st.N evertheless,noa bortionsi nF lock Dw ereobs erveddur ingt hatt ime.T hee wes from FlockE (n=63)s ufferedf romc hlamydial abortionsf orm any years.T hel astc onfirmed case ofc hlamydiala bortionw asdoc umentedi n spring2005. Int hisf lock,s electeds heep(n=13)t hatw eren egativeb y ac ELISAs creeni n autumn2005w erev accinatedw itht hel iveva ccinea ccordingt ot hei nstructionsof t he manufactureri nw inter2 005a ndt estedt wot imesa fterv accination(spring/autumn2006) . Thes erologyof t henon -vaccinateds heepi nt hisf lock(n=50)w asa lsof ollowed.D uringt he investigations,noa bortiondue t o *Cp.abor tus*w asobs ervedi nt hisf lock.

Bloods amplesw erec ollectedf rome achf lockdu rings pring anda utumnof 2005a nd2006 usingV acutainert ubesB ectonD ickinson,H eidelberg,G ermany). Fourhou rsa fter collection,

bloods amplesw ere centrifuged at3000× gf or10 minutesa nds toredi nN uncC ryoTubes (NalgeN unc International,R oskilde,D enmark)a t -20°Cunt ilf urtherpr ocessing.

cELISA

Serums amplesw eret estedb yt hec ompetitivee nzyme-linkedi mmunosorbenta ssay (cELISA)us ingt hem onoclonala ntibodym Ab18 8di recteda gainstt heva riables egments1 (VS1)a nd2(VS2)o ft hem ajorout erm embranep rotein(MOMP)of *Cp.a bortus*, according tot hepr otocolof S alti-Montesantoe ta l.[17].T her esultsof t hec ELISA weree xpressed as ’percentageo fi nhibition’c orrespondingt ot hea ntibodyc oncentrationi nt hes ample. Inhibitionva luesa bove5 5pe rc entw erec onsideredpos itivef ori nfectionw ith *Cp.abortus* (positivec ut-off) whereasi nhibitionva luesbe tween30–55pe r centw ere classifieda s questionable,a ttributablet oe ither *Cp.abortus*or *Cp.pecorum*,a w idelydi stributedc hlamydial agenti ns mallr uminantsc ausingdi seasess ucha s arthritis/conjunctivitisa ndpne umonia syndromei nl ambsa nd alsos ubclinicali ntestinali nfections[18,19]. Inhibitionva luesbe low 30pe rc entw erea ssumedt obe ne gative[17,24].

PCR of eye swabs

Conjunctivals wabs(Cytobrushes,B erdatC harles,B ourroux,S witzerland)w ere collected fromF lockE b eforea nd afterv accinationt oi nvestigatepos siblee xcretion ofc hlamydiae and/ort he *Cp.abor tus*va ccines traint hrought he eye. Before applicationof theva ccine, conjunctivals wabsf rom everys heepi nt hef lock (n= 118) w erec ollectedi na utumn2005. Fivem onthsf ollowingv accination(spring2006),t hes econdc onjunctival swabs amplesw ere takenf rome verys heepi nt hef lock(n= 118).C ytobrushesw eree achpl acedi na 1.5- ml Eppendorft ube ands toreda t- 80°Cunt ilf urtherp rocessing.D NA extractionf roma lls wabs waspe rformeda sd escribedpr eviously[25]us inga c ommercialD NAe xtractionki t(DNeasy TissueK it®,Q iagen, Hombrechtikon,S witzerland).

Thec onjunctivals wabs werei nvestigatedf ort he presenceof c hlamydialD NAb ya *Chlamydiales*-orders pecificP CRt argetingt hei ntergenics pacerr egion(IGS)be tween chlamydial16S a ndt he2 3Sr RNAg enes[26]a ndus ingpr imers cIGS1f(5'-CAAG GTG AG GCTG ATG AC-3')a nd cIGS2r(5'-T CGC CTK TCA ATG CCA AG-3').P CRc onditions arede scribed elsewhere[26].T hei dentityo fa llp ositivelyt ested IGSP CRpr oductsw as determinedb ydi rects equencingof t heP CRpr oductf rombot hs trands.S equencingw as performed witha nA BIP rism377D NAs equencer(AppliedB iosystems)or A pplied Biosystems3100(SynergeneB iotech).T heobt aineds equencesw erec omparedw itht he sequences availablei nG enBankus ingt he BLASTs erver fromt heN ationalC enterf or Biotechnology Information[27].

Investigation of abortion cases

Abortion casesi nt hef locksw eref urtheri nvestigatedf ort hep resenceof chlamydiaeb y routineba cteriology and immunohistochemistryo ft hepl acentaa ndt hef etalor gans(lung, liver,ki dney)a sde scribede lsewhere[28].

Statistical analysis

EweE LISAa ntibodyva luesw erei nitiallyc ategorizedi ntopos itive,que stionableor ne gative asde scribedp reviously[17,24].F ort hea nalysis, questionablea ndne gativer esultsw erebot h interpreted asne gative. Wholef lockr esponsepa tternsove rt imew erevi sualizedus ingbox plots.F ort hoses heept hatw eret esteda llf ourt imes,t hepr oportionof po sitivee wesa te ach timepoi ntw asc omparedw ithine achf lockus ing aF ishersE xactT estw ithe xactp- values. In addition,t hem eant itersof t hoses heepw ere comparedus inga repeatedm easures ANOVA witha nimal ID,t ime (withina nimalr epetitionf actor),va ccinations tatus(flocksB a nd E only),a ndt hei nteractionbe tweent imea ndv accination(againonl yf or flocksB a ndE).

Dataw eres tored andha ndledi nM SE xcel,a nda nalysedus ingt hes tatisticals oftware packagesN CSS2004[29]a ndS PSS14[30].T heove ralll evelof s tatisticals ignificancew as sett o0.05.

Authors' contributions

AGc arriedout t hes erums amplinga ndt hes erologicali nvestigationsa ndd raftedt he manuscript.R Tpe rformedt hei nvestigationof t he abortionc ases andc ontactedt hef lock owners.E V andE Ppr eparedt he cELISApl ates. CKi nvestigatedt he eyes wabsb yP CR. MGDpe rformedt hes tatisticala nalysis.D RZp erformedt heD NAs equencing.A Pa ssistedi n thedr aftinga nd editingo ft hem anuscript.A POS andN Bp articipatedi nt hede sign and coordinationof t hes tudy.A lla uthorsr ead anda pprovedt hef inalm anuscript.

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27.B LAST:B asic LocalA lignmenta ndS earch Toolf romt heN ationalC enterf or Biotechnology Information[www.ncbi.nlm.nih.gov/BLAST].

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29.N CSS,P ASS& G ESS:S tatistics,G raphics,P owerA nalysis,S ampleS ize,& MicroarrayA nalysis[www.ncss.com].

30.S PSS,D ataM ining,S tatisticalA nalysisS oftware, PredictiveA nalysis, PredictiveA nalytics,D ecisionS upportS ystems[www.spss.com].

Figure legend

Figure 1 - Box plots of cELISA antibody values of all examined sheep over the four investigation dates.

Someor a lla nimalsi nf locksA ,B andE w ereva ccinated atg ivent imes(graybox es).

Tables

Table 1 - Serological results A, B, C, D and E

cELISApos itive(above cutoff)s heepw ithf requency, respectivepr oportion(%), mediant itersa ndt iterr ange.

Flock(n)	Parameter	Spring2005	Autumn2005	Spring2006	Autum2006
A	No.pos itive	15	13	11	11
15s heep	Prop. Pos.(%)	100	87	73	73
	Mediant iter	91.7	86.6	81.3	82.3
	Titerr ange	70.9 –99.9	55.1–99.2	62.0–99.0	55.3–99.2
B	No.pos itive	8	4	2	8
26s heep	Prop. Pos.(%)	31	16	8	31
	Mediant iter	65.0	62.9	58.7	63.9
	Titerr ange	61.3 –72.8	55.1–68.1	56.4–61.0	55.3–77.5
C	No.pos itive	14	13	14	15
17s heep	Prop. Pos. (%)[1]	82	76	82	88
	Mediant iter	82.9	72.2	71.3	76.8
	Titerr ange	69.1 –95.2	55.1–89.6	57.1–94.8	55.4–95.8
D	No.pos itive	13	21	29	19
63s heep	Prop. Pos.(%)	21	33	46	30
	Mediant iter	69.5	69.1	69.4	74.3
	Titerr ange	55.1 –100	55.1–93.4	55.6–88.6	56.9–95.2
E	No.pos itive	26	24	19	24
63s heep	Prop. Pos.(%)	42	38	30	38
	Mediant iter	73.4	81.2	80.4	81.8
	Titerr ange	57.4 –94.8	56.5–96.3	55.2–97.6	56.0–98.7

[1]significantdi fferencei n %pos itive(FishersE xactT est,p= 0.024)

Table 2 - Serological results vaccinated vs. non-vaccinated (Flock B and E)

Comparisonof c ELISA positive(abovec utoff)v accinateda ndna turally exposed sheepw ithf requency, respectivepr oportion(%), mediant itersa ndt iterr ange.

Flock(n)	Parameter	Spring2005	Autumn2005	Spring2006	Autum2006
B[1]	No.pos itive	6	3	2	2
14s heep	Prop. Pos.(%)	43	22	14	43
	Mediant iter	62.9	61.3	58.7	64.5
	Titerr ange	61.3 –64.2	55.1–66.3	56.4–61 .0	55.3–77.5
B[2]	No.pos itive	2	1	0	2
12s heep	Prop. Pos.(%)	17	8	0	17
	Mediant iter	71.1	68.1	-	62.2
	Titerr ange	69.4 –72.8	-	-	55.9–68.5
E[1]	No.pos itive	1	0	0	1
13s heep	Prop. Pos.(%)	8	0	0	8
	Mediant iter	61.4	-	-	73.2
	Titerr ange	-	-	-	-
E[2]	No.pos itive	25	24	19	23
50s heep	Prop. Pos.(%)	50	48	38	46
	Mediant iter	76.3	81.2	80.4	82.2
	Titerr ange	57.4 –94.7	56.5–96.3	55.2–97.6	56.0–98.7

[1]vaccinated group

[2]non-vaccinated group

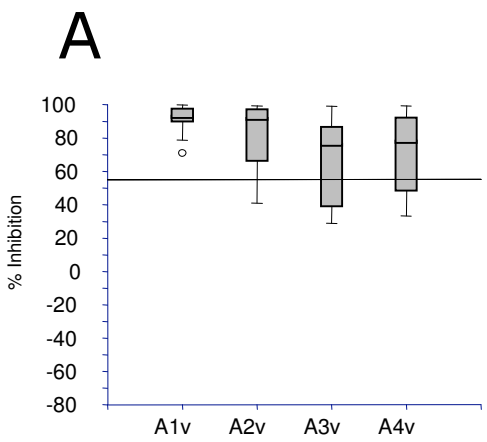
Table 3 - Flock details

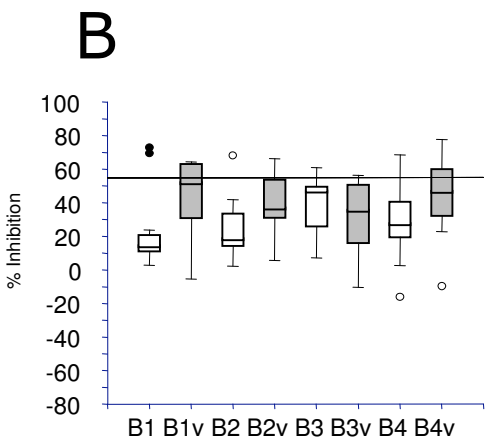
Flock	Examination dates	Average no.s heep	Sheep tested all4 times	Flockhi story	OEA status[1]	Vaccination withl ive vaccine
A	spring & autumn 2005/2006	54	15	chlamydial abortionsi n autumn2004	positive	15s heep (spring2005)
B	spring & autumn 2005/2006	48	26	chlamydial abortion outbreakne arby in2003	negative	14s heep (spring2003), nova ccination booster
C	spring & autumn 2005/2006	45[2]	17[3]	chlamydial abortions (positive control)	positive	no
D	spring & autumn 2005/2006	105	63	noa bortions (negative control)	negative	no
E	spring & autumn 2005/2006	118	63	chlamydial abortionsi nt he past	positive	13s heep (winter2005)

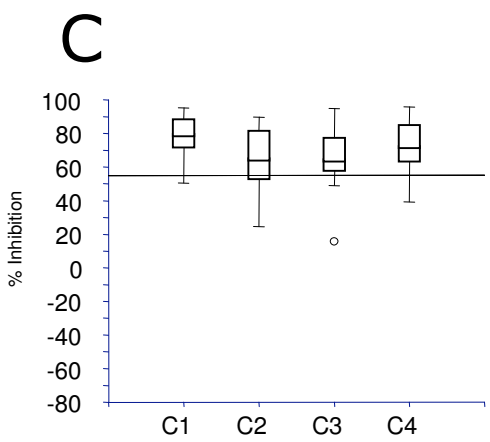
[1]OEA=ovinee nzootica bortion

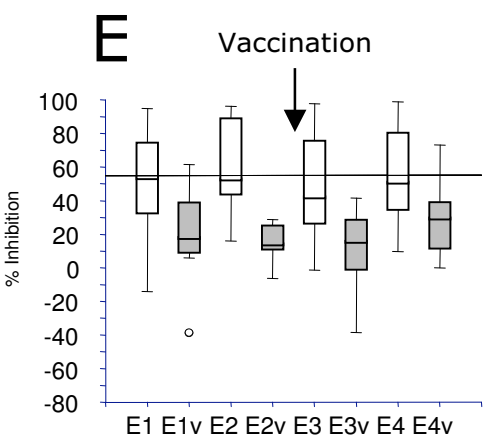
[2]Averageno. goats:11

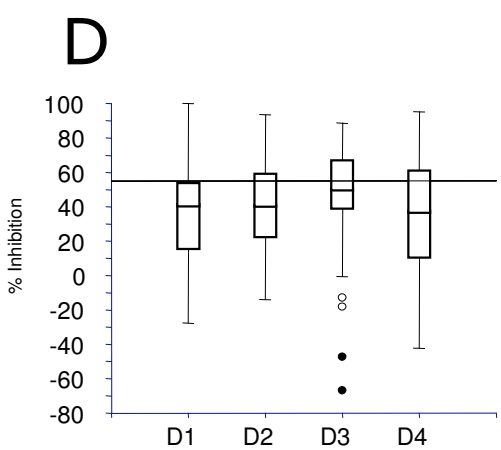
[3]Goatst esteda ll4t imes: 4











Symbol legends

Box plot with 25th, 50th and 75th percentiles, and whiskers representing ~ 95% of the range

Moderate and severe outliers

A1v Flock A, examination 1, vaccinated

Figure 1